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SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.

EXAMINER

ART UNIT | PAPER NUMBER

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Prof. Caprio (Inventor) (3) John Peabody III

(2) Mr. Naylor (4) Marianne Cintins

Date of interview 3-26-92

Type: ☐ Telephonic ☐ Personal (copy is given to ☒ applicant ☒ applicant's representative).

Exhibit shown or demonstration conducted: ☒ Yes ☐ No. If yes, brief description:

Video Tape - showing snapping/biting response / feeding searching behavior.

Agreement ☐ was reached with respect to some or all of the claims in question. ☒ was not reached.

Claims discussed: All

Identification of prior art discussed: That cited in action

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:

1.) Relevance of electrophysiology on predicting actual feeding behavior (injection)

2.) discussed lure v.s. bait language in claim 1.

3.) Exam. indicates reconsideration of art rejection in view of applicants showing of an unobvious result (Papers + Video)

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1–7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☒ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

John D. Peabody III
Examiner's Signature

PTOL-413 (REV. 1-84)

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Submitted during Interview (F-3)
3-26-92

THE UNIVERSITY OF TENNESSEE
MARTIN

See Last few Pages: Pond Cages

F3 Junk Mixture

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FINAL REPORT

Catfish Fry to Fingerling
Feed Flavor Studies
July 16 - November 2, 1991

General:

Jenny Davis' final report (as a weekly account) is enclosed in its entirety; significant observations are recorded below. As you know, the study started later than anticipated but ran the full 100 days for Phases I & II. All flavored feeds out-performed their controls when no supplemental feed was available (vats vs cages in ponds). These data suggest that the fry to fingerling time period, in a nursery-type environment, is aided by the addition of a flavored feed. It would be interesting to note - but not an objective of this study - if these fish also suffered less stress throughout the life cycle.

Specific:

Vats and cages in ponds were dramatically different. It was impossible to count the number of fish that we started with in the ponds because of nightfall and the necessity of getting them from the truck into the pond. Only weights were taken at that time and average weights per fish were recorded at the final weigh-in.

Vats:
Data suggest that all flavors out-performed the controls; F1 by 1.67:1; F2 by 1.47:1; and F3 by 1.47:1. Here the feeds were the only source of nutrients.

Cages:
Data suggest that the F3 flavor may have catalyzed additional feeding since, at the second weighing, the fish were growing at a rate of approximately 2:1. In the final analysis, F3 out-performed all others by:

1) a greater weight gain, and

2) the fish were of uniform weight - a positive factor for harvest time.

It will be important to analyze the cost-effectiveness of each flavored feed and project what combinations of these (F1, F2, & F3)

may have on growth rate and survival. Economic data remain the property of the suppliers, but from this point of view, I would suspect that F3 is the most expensive followed by F1 and finally F2. Please let me know if you wish me to conduct this analysis and indicate your consent to supply the costs. Undoubtedly, the stress of the vats is the key applications area for these flavors which is also the critical growth period. Flavored feeds got the attention of the investigator when arriving by truck at the site, which is important to the fish farmer. It should also be noted that all feeds were tested at only one level, the one suggested by the supplier; no attempt was made to optimize the level.

A copy of this report is being prepared for each flavor supplier. The name of the suppliers have been kept CONFIDENTIAL. in the event you feel that a direct contact to another participant is warranted, please feel free to contact me and I'll arrange same.

Any other additional correspondence with me shall remain CONFIDENTIAL.

Sincerely,

Norm Betz

mh
cc: Jenny Davis

FRY TO FINGERLING

FEED FLAVOR STUDIES

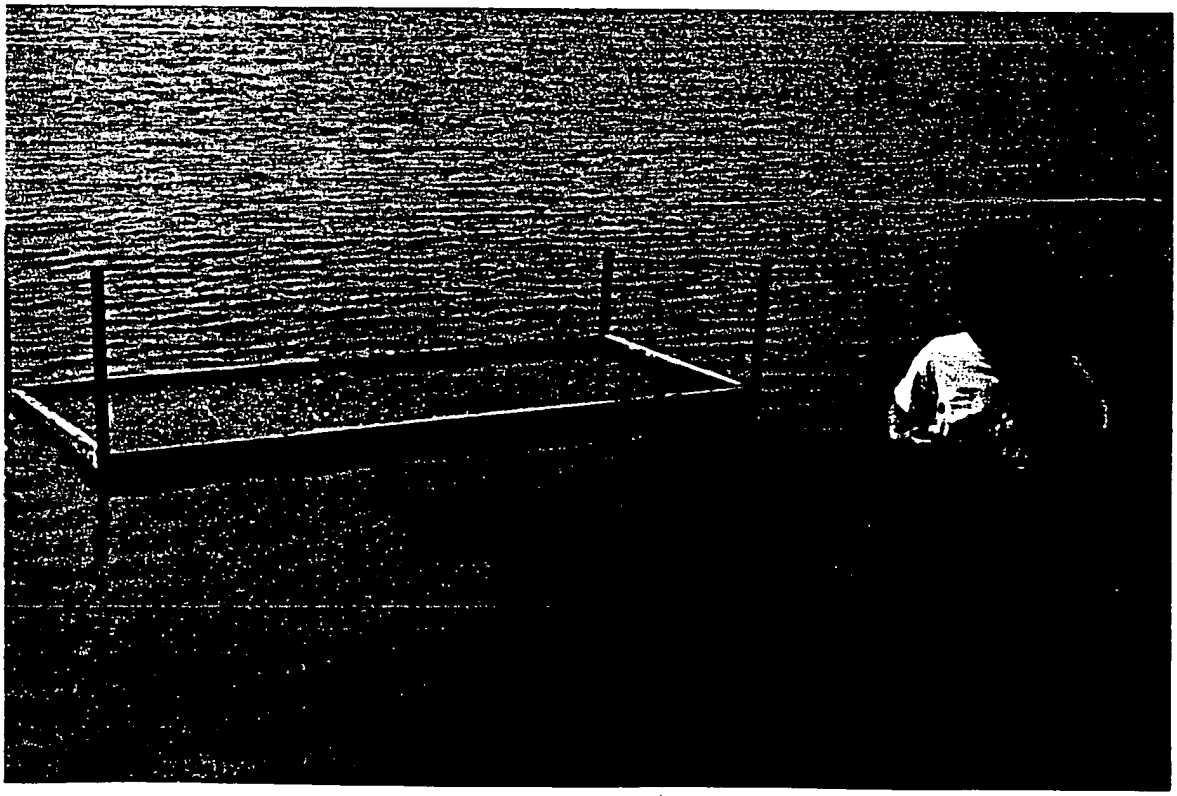
Jenny Davis

November 15, 1991

CONCRETE VATS

CAGES

NOTICE FEEDING BEHAVIOR AT SURFACE!



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INTRODUCTION

The purposes of this research are to minimize mortality and maximize weight gain. The facilities needed for the study were readily available at Ed Davis Fish Farm, and Southern Aquatech of Memphis supplied all of the catfish fry for the experiment.

On July 16, the catfish fry arrived at Ed Davis Fish Farm. They were divided into the proper test groups, with the vats holding 300 fry per test group and with the cages in the pond holding 10.5 pounds per test group (which figures to be approximately 1700 fry). Each individual fry initially weighed 2.2 grams.

FEED PREPARATION

F1 The instructions were for the flavor to be .3% flavor. The flavor was diluted in water (.5% of the weight of the feed). Mixing was done in ten pound increments. The calculations were as follows:

(10.00 lbs.)(.3%)(454 g) = 13.62 g flavor

(10.00 lbs.)(.5%)(454 g) = 227 g water

Therefore, for every ten pounds of feed mixed, 13.62 g of flavor was dissolved in 227 g of water. The solution was then thoroughly mixed in with the feed.

F2 The instructions were for the flavor to be .3% flavor. The flavor was diluted in water (.5% of the weight of the feed). Mixing was done in ten pound increments. The calculations were as follows:

(10.00 lbs.)(.3%)(454 g) = 13.62 g flavor

(10.00 lbs.)(.5%)(454 g) = 227 g water

Therefore, for every ten pounds of feed mixed, 13.62 g of flavor was dissolved in 227 g of water. The solution was then thoroughly mixed in with the feed.

F3 This flavor arrived in a container with a fill-line already marked. The container was marked as being enough for 50-100 pounds of feed. The flavor was dissolved in water (to the fill-line) and 200 ml of the flavor was added

for every 10 pounds of feed mixed.
In preparing each feed, clean equipment was always used to prevent any chance of the flavors mixing. The feed was then weighed and put in marked containers (as to F1 pond, F1 vats, F2 pond, F2 vats, etc.).

DAY 1 - DAY 12

The fingerlings are now in their new homes. We placed 10.5 pounds (roughly 1700 catfish) in each cage, while the vats have 300 catfish in each test group.

The feeding over the last two weeks has gone well. It seems to me that our results will be most apparent on the caged catfish in the pond. At the present time, all of the test groups are getting fed twice a day. The caged groups are getting 75 grams per feeding, and the groups in the vats are getting 15 grams per feeding.

The test groups in the pond responded minimally at first. The F1 test group responded the most vigorously. F2 and F3 showed the secondmost activity. The control group did not respond much at all. After a few days, all groups started responding much more quickly at about the same rate. However, F3 seemed to excel just a bit. After a week of feeding, the fish in all groups seemed to be waiting for me at feeding time.

The test groups in the vats did not show any response for the first couple days. The fish then seemed to swim to the feed, but they never surfaced to eat. It appeared as if they were waiting for the feed to sift down to them. Finally on day 5, I saw response. It was not vigorous, but at least they were surfacing to eat.

In these first two weeks of testing, I am aware of 12 fingerlings dying. I am confident that this was due to some damage suffered in shipping and handling.

DAY 13 - DAY 26

I increased the amount of feed given to the caged catfish from 75 grams to 90 grams starting on day 13. I did not increase the vats' amount of feed because they were not showing much interest at that time. It also appeared as if there was alot of uneaten food on the bottom of the vats.

The fish in the vats finally started to show much more interest in the feed. I cannot really pick one group to be the most active, because it varies from day to day. F1 and F3 have been waiting in the corners at feeding time. Since my last report I have noticed two dead in the F2 group in the vats.

I have really noticed some growth in the caged catfish. At feeding time they are VERY active--often surfacing before I even get the feed in the water. If I had to choose, I'd say F1 and F3 were the most active.

Now, I have some bad news. Birds have been noticed staying close to my cages. It is possible that they might be helping themselves to the fingerlings. The birds are not the only problem. This morning I fed as normal; everything showed its usual, very vigorous, response. This evening when I fed, I had absolutely NO response in the caged F3 group. I checked the cage for holes and found none. It seems as if they have vanished. I will for sure keep you updated.

DAY 27 - DAY 41

On day 31 (August 16),we weighed random samples of each test group. We figured that each individual fingerling originally weighed approximately 2.4 grams. Our results were as follows;

	VATS	CAGES
CONTROL	3.9 g	4.4 g
F1	4.0 g	5.0 g
F2	3.8 g	4.1 g
F3	5.1 g	no sample

It should be noted that the F3 test group from the vat has some sort of algae growing in it. It is possible that the fish are getting some extra nourishment from it.

I once again increased the amount of feed. Starting on day 32, the fish in the vats received 40 g/day, and the cages received 270 g/day.

Response in the vats varies. For five or six days activity is very vigorous; it then slacks off for a couple of days. Overall, F3 shows the most response -- followed by the F1 and control groups. F2 has the weakest response.

With the exception of the F3 group, the caged catfish are doing well. If I had to order them from most to least activity, it would be F1, F2, control, and F3.

It seems as if my F3 group in the cage is toying with me. On day 28, it appeared as if the whole test group was once again eating -- but not vigorously. I did not have a response from them again until day 33 - 34. They once again showed no response for several days. However, since day 37 they've been eating vigorously every day.

DAY 42 - DAY 56

We've started classes at UTM and I feel very displaced from my research! The men that work for Dad are seeing that the fish are getting fed. I'm feeding on Wednesdays and weekends if I make it home.

Activity in the vats varies. For a couple of days it is lively and vigorous; it is then absent for two or three days. It seems as if there has been little or no activity here lately.

Activity in the pond is still VERY strong. More often than not the fish surface for feed when the trucks pulls up. F3 has returned to normal and is very active, just as the other groups are. I've noticed alot of growth in all of the test groups in the cages, but F3 has really excelled.

DAY 57 - DAY 70

We once again took some more random sample weights. Results were as follows;

	VATS	CAGES
CONTROL	4.07 g	5.08 g
F1	4.84 g	6.15 g
F2	4.60 g	6.48 g
F3	6.00 g	9.72 g

Due to the tremendous weight gain of the F3 group, I increased the amount of feed. The vats now get 60 grams/ day, and the cages now get 500 grams/day.

The catfish that are in the vats have been showing little or no activity over the last two weeks. I've also had quite a few die on me over the last two weeks. It's very possible that we stirred them up from the bottom when we were gathering samples.

On the other hand, all four test groups in the pond are still very active. F1 and F2 appear to be growing in the pond and are still very active. F1 and F2 appear to be growing very fast, but they do not begin to compare with F3. The control group is growing, but not quite as rapidly.

DAY 71 - DAY 84

Not much has changed since my last report. Activity in the vats is still at a minimum; it's probably due to the change in the weather.

The test groups in the cages are still showing a very strong response. Overall, I'd have to say that the F2 group has the strongest activity, but it varies from day to day. F1 and F2 have shown some tremendous growth over the last two weeks. The control group is still very active, but it just does not have the size that the test groups do.

DAY 85 - DAY 101

For the most part, this report is the same as the last. There is still practically no response whatsoever in the vats. I think we need to get the fish out of the vats and get their final weight pretty soon. There has been no response from them for quite some time now. I'm afraid that they might regress and lose weight if they remain this inactive for much longer.

The caged test groups, however, are still showing a strong, lively response. The control group has really put on a lot of size. It also appears as if F1, F2, and F3 are getting reasonably close in weight. We'll know for sure pretty soon; I'm anxious to see the results!

TABLE I

FEEDING ACTIVITY -VATS

	DAYS 1-41	DAYS 42-70	OVERALL
MOST ACTIVE	F3	F3	F3
LEAST ACTIVE	F2	F1	F1=F2

TABLE II

FEEDING ACTIVITY -CAGES

	DAYS 1-26	DAYS 27-41	DAYS 42-70	DAYS 71-84	OVERALL
MOST ACTIVE	F1=F3	F1	F3	F2	F3
LEAST ACTIVE	CONTROL	F3*	CONTROL	CONTROL	CONTROL

* F3 is the only group of the cages that experienced an inactive period. For about 10 days F3 showed absolutely no response; other than those few days, they showed a very vigorous response.

TABLE III

RESULTS -VATS

	# OF FISH	PERCENT MORTALITY	INITIAL WEIGHT	FINAL WEIGHT	DIFFERENCE FISH
CONTROL	294	2%	720g	1021.5g	1.03g
F1	280	7%	720g	1589g	3.10g
F2	294	2%	720	1475.5g	2.57g
F3	290	3%	720	1475.5g	2.61g

TABLE IV

RESULTS -CAGES

	# OF FISH	PERCENT MORTALITY	INITIAL WEIGHT	FINAL WEIGHT	DIFFERENCE FISH
CONTROL	1649	.1%	4767g	13620g	5.37g
F1	1836	.2%	4767g	14528g	5.32g
F2	1684	.1%	4767g	12598.5g	4.65g
F3	1631	.2%	4767g	17365.5g	7.72g

TABLE V

WEIGHT ANALYSIS PER FISH AT EACH WEIGHING -VATS

	SAMPLE 1 WEIGHT	SAMPLE 2 WEIGHT	WEIGHT FINAL
CONTROL	3.9g	4.07g	3.47g
F1	4.0g	4.84g	5.68g
F2	3.8g	4.60g	5.02g
F3	5.1g	6.00g	5.09g

TABLE VI

WEIGHT ANALYSIS PER FISH AT EACH WEIGHING -CAGES

	SAMPLE 1 WEIGHT	SAMPLE 2 WEIGHT	WEIGHT FINAL
CONTROL	4.4g	5.08g	8.26g
F1	5.0g	6.15g	7.91g
F2	4.1g	6.48g	7.48g
F3	-	9.72g	10.65g

*All samples were random except for the final weights.

TABLE VII

WEIGHT GAIN PER FISH VS CONTROL PER FISH -VATS

	SAMPLE 1 WEIGHT	SAMPLE 2 WEIGHT	WEIGHT FINAL
F1/C	1.03	1.19	1.67
F2/C	.97	1.13	1.47
F3/C	1.31	1.47	1.47

TABLE VIII

WEIGHT GAIN PER FISH VS CONTROL PER FISH -CAGES

	SAMPLE 1 WEIGHT	SAMPLE 2 WEIGHT	WEIGHT FINAL
F1/C	1.14	1.21	.96
F2/C	.93	1.28	.91
F3/C	-	1.91	1.29

CONCLUSION

Another observation of secondary importance may be the perceived excitement of the caged test groups feeding time. The noise of the truck often stimulated the test groups to excitedly surface for feed. The control group showed a similar response, but it was much more delayed.

The conformity of the groups should also be noted. When the fish were being counted at the final weighing, the uniformity in the size and shape of the F3 test group was very noticeable. The F1, F2, and control groups, on the other hand, were very staggered in size and shape. Some appeared to be the same size as the day they arrived, while others were four times the original size. The F3 test group in the cages and in the vats were the only groups to show any consistency in size.

Due to all of the special attention and extra care given to the catfish, mortality was minimal. A good conclusion cannot be drawn on the mortality rate from this research.

All in all, it is apparent that the flavored feed is better than the control feed when weight gain is in consideration. These results will be more meaningful when an economic analysis of the supplements is prepared.

See fig 1

Papers showing 42 obvious result from Interview, 3-26-92

Physiology & Behavior, Vol. 19, pp. 743–747. Pergamon Press and Brain Research Publ., 1977. Printed in the U.S.A.

Conditioned Aversion to Amino Acid Flavors in the Catfish, *Ictalurus Punctatus*

EDWARD E. LITTLE

Department of Biological Science, Florida State University, Tallahassee, FL 32306

(Received 27 November 1976)

LITTLE, E. E. *Conditioned aversions to amino acid flavors in the catfish,* Ictalurus punctatus. PHYSIOL. BEHAV. 19(6) 743–747, 1977. – Naive catfish readily accepted food flavored with L-cysteine but generally avoided foods flavored with other amino acids. The aversions to these latter amino acids quickly disappeared after the fish had feeding experience with each. When illness induced by lithium chloride was paired with an amino acid, that flavor was strongly avoided. Amino acids structurally similar to that were also avoided while other amino acid flavors were readily consumed. My studies on anosmic fish indicate that olfaction does not play a role in conditioning amino acid taste aversions. Observations of the sequential units of the feeding response suggest that sensory input to the vagal lobe of the brain is primarily responsible for taste aversion discrimination.

Feeding behavior | Conditioned taste aversion | Amino acids | Lithium chloride | Catfish | *Ictalurus punctatus* | Olfactory tract section | Medulla oblongata

WHEN ingestion of a food material is followed by illness, subsequent feeding responses become modified such that the food paired with illness is avoided during feeding. Conditioned taste aversion or bait-shyness has been found to occur in a diversity of vertebrates including fish [9].

In rodents the salient bait-shyness stimulus, flavor, appears to be mediated predominantly if not solely through gustation [7]. In fish such distinctions are more ambiguous since olfactory and gustatory stimuli both occur in aqueous form.

Fish have three anatomically distinct chemosensory channels of which at least two are highly developed in catfish. The common chemical sense [10] will not be discussed further. Olfaction is limited to the nasal lamellae where densely packed receptors, comprising the olfactory mucousa, send axons to the forebrain. Gustation is made up of two submodalities. Taste buds which profusely cover the catfish's body project input to the facial lobe of the medulla oblongata (Facial Taste System), while taste buds in the mouth and gill arches are anatomically linked to the vagal lobe of the medulla (Vagal Taste System) [8].

The chemical senses of catfish are particularly acute. Their olfactory and taste receptors are especially sensitive to amino acids, which are believed to be a major stimulus signalling food [2,11]. Moreover, the study of fish may yield evolutionary insight into the development of the chemical senses and their role in baitshyness behavior.

In the following, results of studies on the channel catfish's preference for foods containing single added amino acids and how their preferences are modified as a result of lithium chloride toxicosis are reported. The roles of olfaction and the facial and vagal taste systems in the bait-shy response are also discussed.

PROCEDURES

Thirty channel catfish weighing 0.5–1.0 kg were obtained locally and were held separately in 100L aquaria throughout the study. The aquaria were supplied with shelters for the fish to hide in and were rinsed with a constant flow of fresh water (1.5 L/min) which ranged in temperature from 17° to 21°C. Since fish diseases and common treatments for these often affect chemosensitivity and feeding behavior, prophalactic treatment was not used and fish were dropped from the study at the first sign of infection.

The fish were fed a food-gel containing 200 g water, 50 g powdered meal (Purina Trout Chow), and 7 g gelatin. The flavor stimuli for preference tests and taste aversion experiments were prepared by adding 0.1 g amounts of single amino acids to the food-gel to form a 4×10^{-3} molar concentration. By using this basic food-gel the flavor of the gel could be changed without altering color or texture. The amino acids, L-alanine, L-arginine, L-glutamic acid, L-cysteine, L-methionine, L-proline, and L-serine were used as flavor stimuli in preference tests. Methionine, cysteine, and serine were used in the taste aversion studies.

During daily feeding sessions the fish were given 1.0–3.0 g cubes of unflavored food-gel. The cubes were presented by dropping them into the fish's tank near the opening of the shelter. The fish were given ten min to eat the cube during each presentation. Uneaten cubes were removed and five min later a second cube was offered. The amount of food-gel cubes presented each day was increased to determine the maximum amount of food that was eaten consistently by each fish.

Flavor preference tests were started after consistent

feeding rates had been found for each fish. Each amino acid flavor was given to the fish separately but in equal amounts. The order of amino acid presentations was randomized during each session. Two groups of four fish were tested for their response to various novel amino acid flavors. The size of one group was enlarged to ten fish and these were tested to determine if their response to novel amino acid flavors changed over time.

The same procedures were followed in preparing other groups of fish for conditioned taste aversion experiments. Food-gels containing amino acids were given to the fish prior to aversive conditioning until 100% of each was consistently eaten. This was done to insure that a reduced preference for an amino acid flavor would be a result of the conditioning and not neophobic aversions.

Lithium chloride (LiCl) was added to the food-gel flavored with one of the three amino acids and fed to the fish. Only one amino acid flavor was given to the fish during this single aversive conditioning session. The amount of LiCl added to the food-gel was adjusted to the fish's normal feeding rate such that each fish received a dose of 0.5 g LiCl/kg body weight. This method of applying LiCl was used rather than injection because catfish will not eat for up to 10 days after any degree of physical handling.

Testing sessions for flavor aversions were begun on the day following the LiCl–amino acid pairing. The fish were given LiCl paired and unpaired amino acid food-gels as during the pretraining sessions. Four fish that had been treated like the test fish but had not been given LiCl were used as controls.

The response of orientation to, barbel contact with, and ingestion of each amino acid was recorded from a limited number of fish whose behavior was not inhibited by observation. The amount of flavored food-gels consumed by each fish was measured. Dunnett's test for multiple comparisons was used to determine differences between the response of LiCl conditioned and control fish to these flavors. *t*-Tests for related samples were used to determine the statistical significance of the difference in the response made to the amino acid flavors after LiCl conditioning.

In preparing anosmic fish, 2–3 mm sections of both olfactory tracts were resected after they had been exposed surgically by cutting a flap of skin along the dorsal midline immediately anterior to the eyes and a small hole had been bored through the overlying bone. The fish were anesthetized with MS–222 (Tricaine methanesulfonate; 1:6000 dilution) and their gills were perfused with water throughout the surgical procedure.

RESULTS

Preference Tests

Food-gels containing cysteine were strongly preferred over those containing other novel amino acids and the plain food-gels during flavor preference tests (Fig. 1a). In some cases L-methionine and L-alanine were eaten as readily as the plain food-gel, whereas gels containing amino acids such as proline, glutamic acid, serine, or arginine were rarely eaten during the fishes' first exposure to them.

The fishes' response to these novel flavors greatly increased after repeated experience. Preferences for or aversions to any particular amino acid were no longer evident (Fig. 1b). The response to serine, for example, increased to 100% over 5 feeding sessions. Thus the neophobic responses (aversions) to amino acids are temporary and tend to disappear as soon as the fish have eaten the novel amino acid flavor at least once.

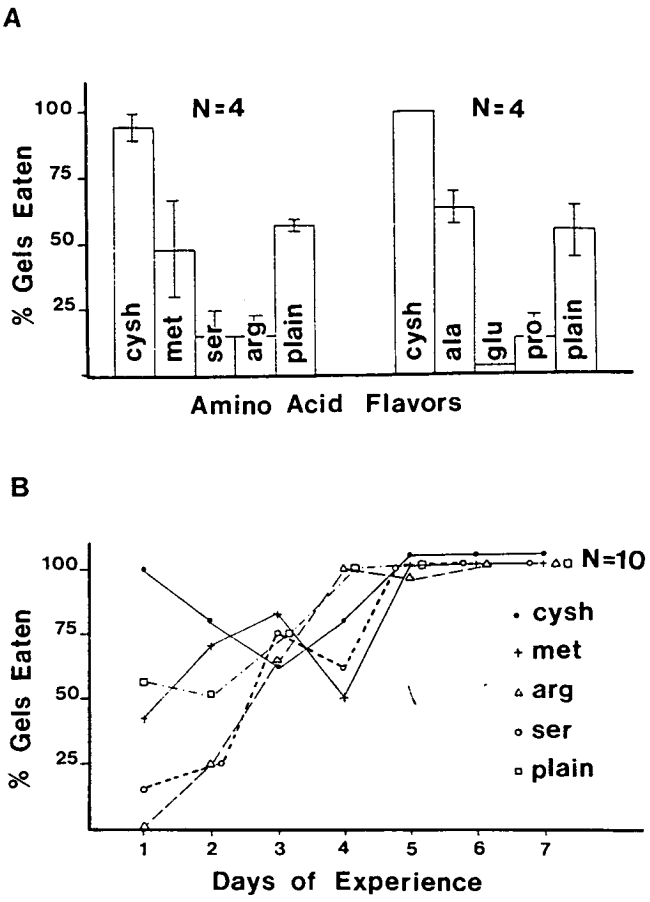

FIG. 1. (A) Naive fish were given food gels containing the following single amino acids: cysteine (Cysh), methionine (Met), serine (Ser), arginine (Arg), alanine (Ala), glutamic acid (Glu) and proline (Pro). No additional amino acids were added to the plain gel. Standard error of response is indicated on each histogram bar. (B) Fish were given food gels containing different amino acids in daily feeding sessions over 7 days. The neophobic responses made initially to different amino acid flavors quickly disappeared.

Lithium Chloride Induced Aversions

The fish ate nearly 100% of the cysteine flavored food-gels containing LiCl during the single training session of aversive conditioning (Fig. 2a). Their response to food-gels containing LiCl did not differ significantly from their pretraining response to cysteine ($t = 1.5$ $p<0.05$). The effects of LiCl toxicosis were evident within 2 to 4 hr after consumption. The fish became lethargic and unresponsive to light or touch, occasionally the fish lost equilibrium and were awkward in swimming. Three fish in this group also vomited.

The fish ate very little of any amino acid food-gels on the day following LiCl conditioning. The response to these flavors was significantly lower than both the preconditioning level of response and the response of the control fish. The fishes' consumption of the safe serine food-gel quickly increased to the pretraining level of response during

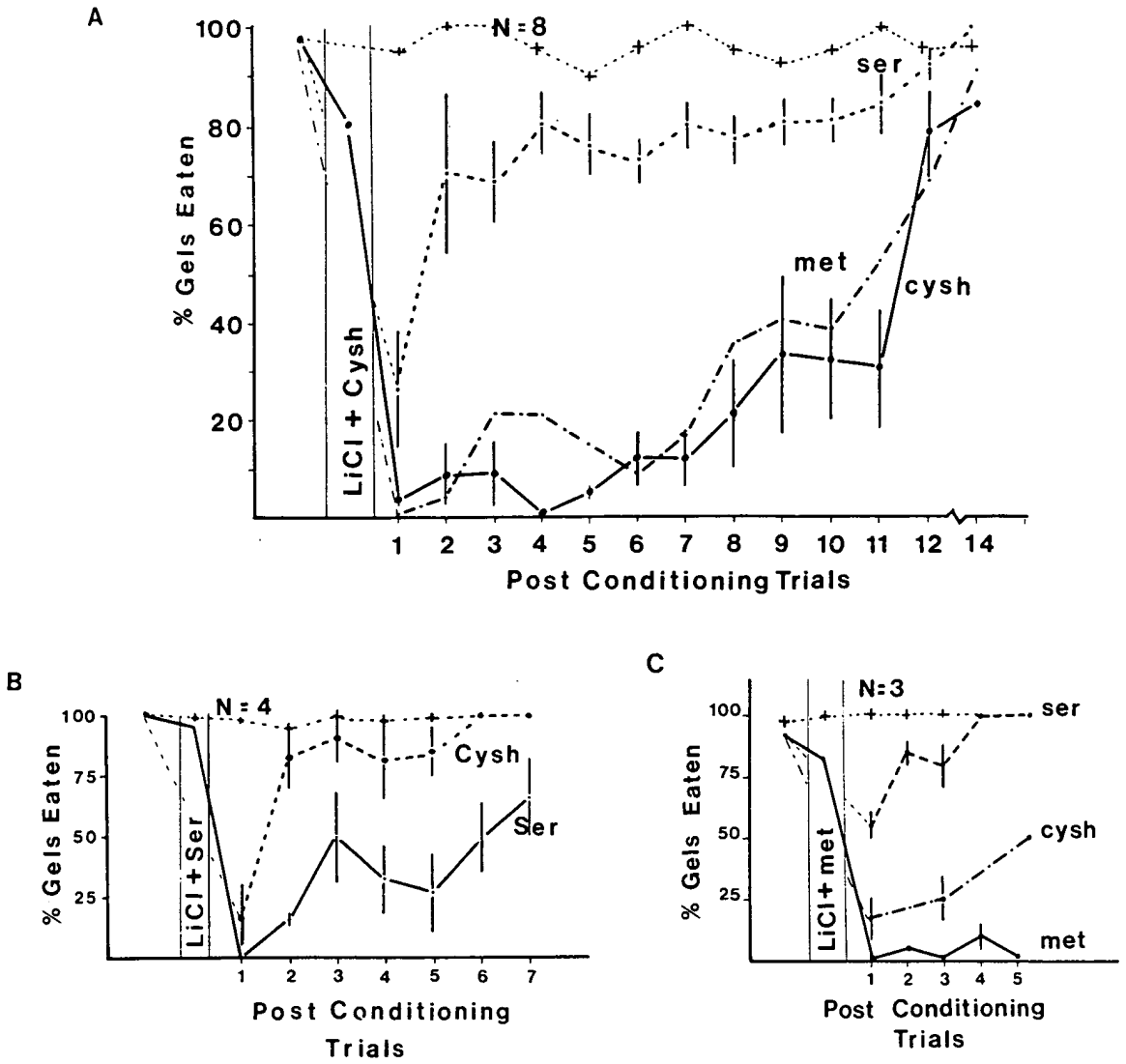

FIG. 2. Fish consumed all serine, cysteine, and methionine flavored food-gels prior to LiCl toxicosis. They also consumed most of the flavored gels containing LiCl. The response to the illness paired flavor was significantly reduced during the post conditioning sessions. The response of non conditioned fish is indicated by +-----+ in each graph. The standard error of response is indicated at each point. (A) Fish avoided cysteine and methionine flavored food-gels after they had eaten cysteine gels containing LiCl. Results of Dunnett's test indicate that responses to cysteine and methionine were significantly lower ($p<0.05$) than responses made by the control fish until Day 14 of the postconditioning session. With the exception of Day 1, the response of conditioned fish to serine was similar to the response of the control fish. t-Tests for related samples indicate that the response to serine was always significantly higher than the response to methionine and cysteine ($p<0.05$). Responses to cysteine and methionine were never significantly different. (B) Serine was avoided after Ser + LiCl training. The response to serine was significantly lower than that of the control fish (Dunnett's test, $p<0.05$) and differed significantly from the response to cysteine ($p<0.05$). (C) Fish also avoid methionine flavored gels after Met + LiCl training. The response to methionine was always lower than the response shown by control fish (Dunnett's test, $p<0.05$). It was also significantly lower than the response to serine ($p<0.05$). The response to cysteine was not significantly different from the the response to methionine.

subsequent tests. Their posttraining response to serine, with the exception of the Day 1 response, did not differ significantly from their pretraining response or the response of the control group.

Consumption of the illness-paired cysteine food-gel was drastically reduced during the first 11 postconditioning sessions, and was significantly lower than either their pretraining response to cysteine or the response of the control group. Consumption of cysteine flavored gels did not reach preconditioning levels until 14 days after LiCl conditioning.

The fish appeared to have generalized their response to methionine since they avoided it as frequently as cysteine. The response to cysteine and methionine was significantly lower than their response to serine. The gradual increase in the consumption of illness-paired and unpaired amino acids observed during postconditioning sessions strongly resembles the extinction of neophobic responses seen earlier.

Similar taste aversions were seen when either serine (Fig. 2b) or methionine (Fig. 2c) were paired with LiCl. Thus the three amino acids are effective stimuli for conditioning taste aversions, indicating that catfish can discriminate between the flavors of single amino acids.

In other tests LiCl was added to plain food-gels, omitting the amino acid flavor, to determine if the flavor of LiCl was aversive. Plain food-gels were used in subsequent feeding experiments. As in previous experiments the consumption of the plain food-gel was lower during the first postconditioning session but recovered to pretraining levels during the second or third session. The fish did not avoid food-gels containing LiCl when administered at intervals of 8, 6, 4, or 3 days after previous LiCl-induced illness, which indicated that the flavor of LiCl is not particularly effective as a stimulus for conditioning aversions.

Observations of Feeding Behavior

A sequence of responses led to the consumption of an amino acid flavor including an orientation to the food, barbel contact with it, biting or chewing the gel and finally swallowing or regurgitating it. There is an increase in the amount of barbel contact with food-gels of novel flavors and a longer latency before the gel is brought into the mouth. Often the food was repeatedly brought into the mouth and spat out. Essentially the same response was made to illness-paired amino acid flavors, with lengthened periods of barbel contact and of holding the gel cube in their mouths (Fig. 3).

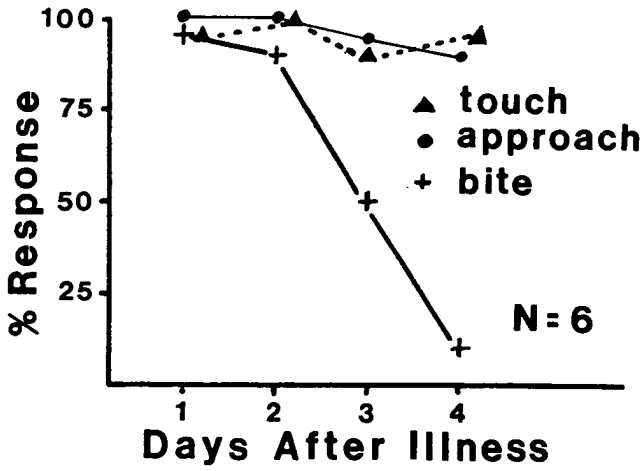

FIG. 3. During the first 2 to 3 days following LiCl illness the fish continued to approach, touch and bite the illness-paired food gel. On the third or fourth day they rejected the illness-paired flavor after touching them and no longer bit them.

The fish rejected the illness-paired food gels only after these had been brought into the mouth, which strongly indicates that discriminations between illness-paired and unpaired flavors are made in the mouth, while stimulation occurring during the approach to the gel and barbel contact with it are not discriminated. However, within the third or fourth day after aversive training the fish continued to approach the gel cubes, but they rejected the illness-paired amino acid flavor after barbel contact and no longer took cubes of this flavor into their mouth.

Conditioned Aversion in Anosmic Fish

Olfactory receptors are sensitive to serine, cysteine, and methionine. When fish were prevented from receiving olfactory input by surgically interrupting the olfactory tracts, their responses to serine, methionine, and cysteine after illness-paired cysteine consumption was essentially the same as found in normal fish (Fig. 4), with serine consumption equaling pretraining rates, while consumption of cysteine or methionine was significantly reduced.

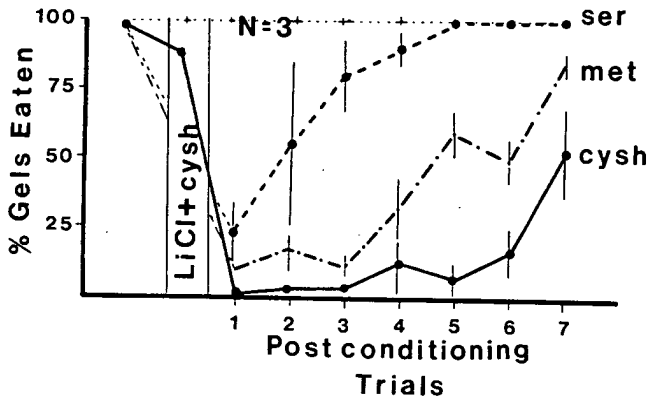

FIG. 4. Anosmic fish readily discriminated between cysteine and serine food-gels after cysteine-LiCl treatment. The response to cysteine was always lower than the response shown by control fish (Dunnett's test, $p<0.05$). The response to cysteine was also significantly lower than the response to serine ($p<0.05$). The response to methionine was not significantly different from the response to cysteine.

DISCUSSION

Individual amino acids have been shown to occur naturally in mixtures with other amino acids as well as other chemicals [3]. It was not known if the fish would respond to or discriminate between single amino acids behaviorally, even though electrophysiological observations had shown that olfactory and taste receptors are extremely sensitive to them. This study has shown that the addition of a single amino acid creates a potent stimulus for behavioral responses and catfish can readily discriminate between foods containing different amino acids. The concentration of the added amino acid was approximately 4×10^{-3} molar, however the identity and concentration of other amino acids existing in the composition of the gelatin and powdered meal was not known.

Channel catfish have omnivorous feeding habits. Their diet includes a diversity of living and decomposing vertebrate and invertebrate tissue and plant material; thus it is of obvious survival advantage that the fish discriminate the flavors of toxic substances from the diversity of flavors that they are likely to encounter in nature.

The catfish in this study appear to have used two strategies to avoid illness. Their neophobic response to novel amino acid flavors is one way by which they could avoid being poisoned. The subsequent consumption of limited amounts of the novel flavors, while still minimizing the risk of illness, would allow the fish to develop preferences for new flavors and thereby broaden the range of potentially beneficial foods in their diet.

A second strategy was to discriminate flavors of beneficial foods from those associated with illness and to avoid the latter [6]. After LiCl illness, the fish continued to eat most amino acid flavored food-gels yet avoided those that lead to illness.

Fish ate little of any amino acid flavor during the first postconditioning session, possible because they were still ill. Alternatively, the response may have been caused by a brief generalized aversion to all food-gels. Rats exhibit a brief aversion to both novel and familiar stimuli when exposed to these after having experienced toxicosis in the absence of a stimulus [4].

Electrophysiological [2] and behavioral studies (Little, submitted) have shown that amino acids of equal concentration differ in their relative effectiveness. Differences in the effectiveness of the amino acids used in the present study did not appear to be an important variable in the present study, since aversions were limited to those flavors that had been paired with LiCl.

Fish responded similarly to cysteine and methionine when either of these was paired with illness. This might have resulted from an inadvertant during the first postconditioning session when the fish were still ill. Were this the case, however, then serine should have also been aversive. A more likely alternative explanation would suggest that the response to methionine and cysteine were a generalization since these contain sulfur and may have seemed similar to the fish.

The amount of experience preceeding illness may be an important variable in the successful formation of taste aversions. Strong aversions to amino acids occurred even though the fish had prior experience with them. This experience was relatively brief in comparison to their experience with unflavored food-gels on which they had been raised. Aversions to illness-paired unflavored gels were of brief duration. Similarly, Domjan [4] has shown that illness failed to disrupt drinking behavior in rats when it was paired with flavors on which they had been reared. He also found that exposure to a flavor for up to 25 days prior to illness produced aversions to that flavor.

Limited observations made during this study indicate that higher concentrations of amino acids or higher LiCl dosages resulted in a lengthened period of aversion as was shown in rats [7]. LiCl was not aversive to fish after repeated toxicosis, possibly reflecting the poor salt sensitivity of taste receptors or dietary deficiencies.

Atema [1] has shown that the initial response to chemical stimulation is an interruption of ongoing activity and that it is mediated by olfaction. The facial taste system directs the fish's orientation to the food source and also mediates biting the food material when stimulated by contact with the barbels or other external body surface. The vagal taste system is stimulated when food is brought into the mouth and directs swallowing if the food is desirable or else rejected. The chemosensory system under stimulation during this discrimination can be implied from the sequential unit of the feeding response during which the food is rejected.

Olfactory stimulation is of little importance in neophobic or bait-shy aversion since normal fish continue to approach LiCl – paired, unpaired or novel flavors. Aversively conditioned anosmic fish readily discriminate between flavors that have been paired with illness and those that are safe.

A further differentiation between the role of the facial and vagal taste submodalities in mediating bait-shy discriminations can also be suggested. During the first 3–4 days following LiCl illness the illness-paired flavor is rejected only after the cube has been brought into the mouth, otherwise the fish continue to approach, touch, and bite it. Discriminations occur only after the gel has been brought into the mouth, which strongly suggests that stimulation through the vagal taste system predominates in directing aversions. During the third or fourth day postillness, there is a shift in the point of the feeding response at which discriminations between flavors are made. The flavor is rejected after barbel contact and is no longer brought into the mouth, suggesting that bait-shy discriminations are now mediated by the facial taste system. Undoubtedly sensory overlap or possibly other anatomical relationships between the facial and vagal lobes [5] are seen behaviorally.

Evolutionary strategy may have linked the facial taste system to orientation and food search in order to maximize the possibility of finding potential food while the vagal taste system, playing a more conservative role evaluates the food further and is ultimately responsible for accepting it.

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MODIFICATION OF CONSPECIFIC CHEMO-ATTRACTION IN ARCTIC CHARR, *SALVELINUS ALPINUS* (L.), BY NITROGENOUS EXCRETORY PRODUCTS

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Abstract—1. The influence of nitrogenous excretory products, L-alanine and ammonium chloride, on the strength of olfactory mediated attraction of juvenile Arctic charr to water scented by two, 10 or 20 conspecifics was studied in a fluviarium.

2. The test fish was strongly attracted to water scented by charr, but with addition of L-Ala to a concentration of 4.6 μM, the water became repellant with two donors, with 10 donors the response varied from one charr to the other, i.e. either attraction or avoidance.

3. Addition of ammonium chloride to a concentration of 5.6 μM in water scented by two donors gave an indifferent reaction, but with 20 donors the same concentration of ammonium chloride gave no reduction in the strength of attraction.

4. Accordingly, it appears that nitrogenous excretion products can modulate the attractive effect of water containing conspecific attractants.

INTRODUCTION

Previous studies showed water scented by Arctic charr [*Salvelinus alpinus* (L.)] to be strongly attractive to conspecifics (Höglund and Åstrand, 1973; Höglund *et al.*, 1975; Selset and Døving, 1980; Olsén, 1985, 1986a; Olsén and Höglund, 1985). A preference–avoidance study was performed with pure amino acid solutions in the same proportions as was observed in water conditioned by the presence of juvenile Arctic charr (Olsén, 1986b). The charr avoided this mixture of 17 L-amino acids. Preference–avoidance tests were also done with only L-alanine or ammonium chloride as stimulus. Both substances were avoided by the charr. L-alanine was tested at the same concentration as the mixture of 17 L-amino acids. Interference by excretion products such as ammonia and amino acids with chemical communication in fish has not been demonstrated, although enhanced concentrations of these substances may have caused the avoidance to water scented by conspecifics observed by Le Martret and Saglio (1982) and Stabell (1982).

The aim of the present experiments was to study the effect of L-alanine and ammonium chloride on the strength of attraction to conspecific odour. This was done by way of the addition of these substances to charr scented water in fluviarium tests. Alanine was chosen as a representative of L-amino acids as in preference–avoidance tests it had an equally strong repellent effect on Arctic charr as the mixture of 17 amino acids (Olsén, 1986b).

MATERIALS AND METHODS

Fish

The behaviour of 67 specimens out of 230 hatchery-reared Arctic charr were studied. They were the progeny of mature fish captured during the autumn, 1981 outside the village of Åbränna on Lake Torrön, in the province of Jämtland, Sweden. In our laboratory all 230 specimens were kept together in a tank supplied with municipal tap water which is rich in calcium (approx. 3 mM). They were kept under illumination from 0007 until 1730 hr and fed Astra-Ewos "Salmon Feed Extra" daily at 0009 and 1500 hr. Mean fresh weights were 12 g (SD: ± 5, range: 7–27, $N = 22$) in October 1983, 16 g (± 10, 4–47, $N = 67$) in November 1983 and finally 42 g (± 17, 14–74, $N = 30$) in May 1984. The total ammonia-N concentration ($NH_4^+ + NH_3$) in the storage tank was analysed using the indofenol method (Chaney and Marbach, 1962) 40 min and 3 hr after feeding. It gave 120 μg/l ammonia-N (10.5°C, pH 7.8) on both occasions. The total ammonia-N concentration in clean tap water was 6 μg/l. The water temperature in the storage tank was 11–12°C in September–October and 7–10°C in April–May.

Apparatus for photo-recording

The fluviarium, described by Höglund (1961), was used as modified by Olsén and Höglund (1985). It was fed by 17.9 l/min of aerated municipal tap water (pH 7.50–7.76, $N = 3$). The water temperature in fluviarium was the same as in the storage tank.

Each of two peristaltic pumps transferred water to each lateral side of the fluviarium at the rate of 300 ml/min from two 12 l aquaria. Each of these aquaria was continuously supplied with 400 ml/min of flowing tap water. In control tests (C tests in Fig. 1) pure tap water was transferred through both aquaria. In tests with charr scented water (A, AP, and AP + A tests in Fig. 1) 2, 10 or 20 odour-donating charr were placed in one of these aquaria.

All tests were run in darkness and started at 1730 hr. A single test fish was placed in the illuminated test area at least half an hour before the first test period. The momentary positions of the test fish were recorded every 150 sec with a 16 mm film camera and the flash light was filtered through a Kodak WRATTEN filter 89B with less than 0.10% transmission at 670 nm and 1.58% at 690 nm (cf. Kodak Filters Publ., 1978). The photo-recording started when the fish was placed in the test area of the fluviarium. The test fish's preference was based on the number of observations in one or the other lateral half of the test area both during consecutive test periods alternatively containing pure water or diverse water qualities. Each test comprised eight simi-

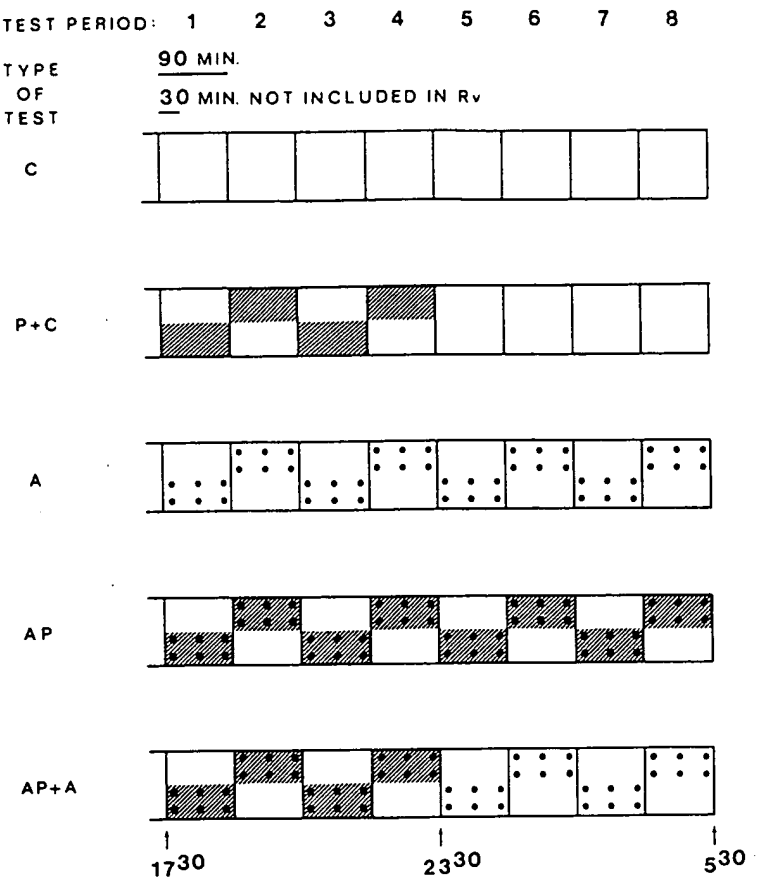

Fig. 1. Type of tests performed. Control tests (C) were performed with pure tap water supplied to both halves of the test area. Dots indicate that charr scented water was added. Shaded areas mean that L-Ala or ammonium chloride was supplied to either side of the fluviarium.

larly or, four plus four differently arranged water conditions as shown in Fig. 1. Between test periods (each lasting for 90 min) the water qualities distributed to one or the other lateral half of the test area were altered automatically by switching two electromagnetic valves. The total number of observations (photo-recordings) in each 90 min period was 36.

Tests performed

The type of tests performed is given in Fig. 1. The abbreviations used there are as follows: C = control conditions, i.e. pure tap water in both halves; A = charr scented water was distributed to one, and pure tap water to the other half of the test area; P = L-Ala or ammonium chloride was supplied to one half of the test area, pure tap water to the other; AP = combination of A and P, i.e. one or the other nitrogenous substance was added to charr scented water distributed along one or the other lateral half.

Tests with eight similarly arranged periods

All tests were done during the period 11 September 1983 to 8 October 1983. Control tests (C tests) were performed with pure tap water added at a rate of 300 ml/min to either side of the fluviarium from two 12 l. donor aquaria. Then at the same time 26 ml/min tap water was pumped from a polyethene tank into the side in addition to water from one of the donor aquaria. Six attraction tests (A tests as stated above) were performed as described by Olsén and Höglund (1985), the only innovation being the supply of tap water (26 ml/min) to the lateral half of the fluviarium containing scent from 20 charr. Five more attraction tests were run with test fish, made anosmic by cauterizing their olfactory epithelia under MS 222 anaesthesia using a fine tip of soldering copper.

During AP tests ammonium chloride was added to the half of the fluviarium containing water scented by 20 charr.

Tests divided into two parts (four plus four test periods)

All tests were performed during the period 5 April 1984 to 29 May 1984. During test periods 1–4 test stimuli or pure tap water from a 13 l. glass beaker (pH 7.46–7.61, $n = 4$) were supplied in parallel to pure tap water or water scented by charr pumped from one of the two 12 l. aquaria. Tap water from an identical beaker was supplied to the other half of the test area always together with pure tap water from the other aquarium empty of fish.

Control tests (C tests) were run with pure tap water from both 12 l. aquaria during all periods, 1–8. Tap water was supplied from the glass beakers during periods 1–4.

Attraction tests (A tests) were performed with two or 10 donors in one of the two 12 l aquaria. Then during test periods 1–4 pure tap water was supplied to each side of the fluviarium from two glass beakers.

In P + C tests one charr was allowed to make choices between water with a low concentration L-Ala and pure tap water during the four initial test periods, 1–4. Then control conditions followed during the periods 5–8, with tap water from both 12 l. aquaria and without any further supply from glass beakers.

Finally, a fourth combined test (AP + A tests) with L-Ala or ammonium chloride supplied during the attraction test in periods 1–4, i.e. with the nitrogenous substance and the charr odour from two or 10 specimens presented on the same half of the test area, then followed by "pure" attraction test conditions during the final periods, 5–8.

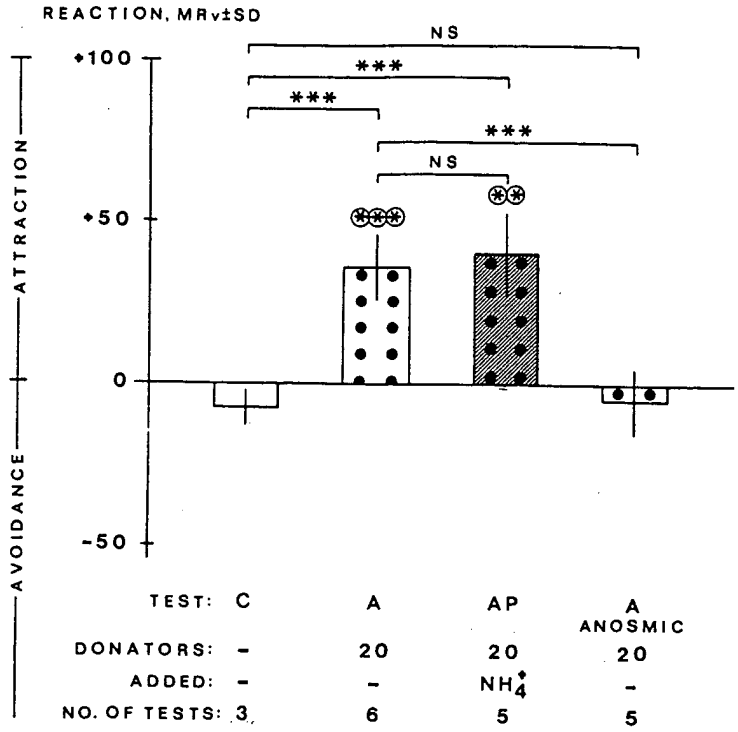

Fig. 2. Attraction in intact and anosmic juvenile Arctic charr to water scented by 20 conspecifics during eight consecutive test periods. AP tests were performed with the addition of 5.6 μM ammonium chloride to charr scented water. The significance levels of $MRv:s$ differing from an indifferent reaction, $MRv = 0$, are as shown by encircled asterisks, otherwise as denoted by brackets and asterisks, using Student's t-test: NS, $0.1 < P$; (*) $0.05 < P < 0.1$; * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$.

Data analysis

A reaction value (Rv) for a whole test or part of it was calculated according to the formula:

$$Rv = [(N_{st} - N_{aq})/(N_{st} + N_{aq})] \times 100;$$

where N_{st} represents the number of observations in the half of the test area containing stimulant(s), i.e. nitrogenous substance and/or charr odour. N_{aq} represents the number of observations on the other half with tap water. The total number of observations, $N_{st} + N_{aq}$, for a test period was 24 as the initial 12 photo-recordings (30 min) of each period were excluded from the calculations of the Rv. The arithmetic mean for $Rv:s$ from identical tests is designated MRv. Student's t-test with the degree of freedom corrected due to differences in variance was used to compare $MRv:s$ from different types of test (Bailey, 1981). A Student's t-test was also used when comparing $MRv:s$ with the theoretical MRv for an indifferent reaction ($MRv = 0$). Comparisons between $Rv:s$ within identical tests were made with a paired t-test. The criterion chosen for a significant difference is $P = 0.05$, although P values between 0.05 and 0.1 are noted.

RESULTS

Tests with eight similarly arranged periods

The results of C, A, AP tests are shown in Fig. 2. Control tests did not reveal any preference for tap water delivered from either aquarium. Intact charr were strongly attracted to water conditioned by conspecifics (A tests) while the response of anosmic charr was indifferent and not significantly different from controls, indicating that the attraction to charr scented water is mediated by the olfactory sense (Höglund and Åstrand, 1973).

The addition of 5.6 μM ammonium chloride (AP test) to water scented by 20 charr had no effect on the strength of attraction.

Tests divided into two parts (four plus four periods)

The results of C, A, P + C, and AP + A tests are shown in Fig. 3. C tests revealed no preference for water transmitted from either 12 l donor aquarium as the MRv for periods 1–4 and periods 5–8, respectively, were not significantly different from nil.

The charr showed in the A tests a strong attraction to conspecific odour and there was no difference in the strength of attraction during the periods 1–4 as compared with 5–8. In P + C tests the charr avoided 4.6 μM L-Ala ($P < 0.01$) but this did not occur at the addition of one-tenth of this amino acid concentration. The response was indifferent during periods 5–8, then there was no supply of L-Ala.

In AP + A tests when 4.6 μM L-Ala was added during periods 1–4 to water scented by two conspecifics, this combination of stimulants was avoided significantly. The same concentration of L-Ala combined with odour from 10 charr gave an indifferent reaction. In that case the great variance of $Rv:s$ in the periods 1–4 depends on two highly negative (−47.2, −46.5) and two highly positive $Rv:s$ (+37.1, +58.6). This may be due to individual reactions of the actual test fishes, i.e. in some specimens the attractive force overcame the repulsive effect of L-Ala. A significant attraction to charr scented water arose anew in test periods 5–8 since the supply of L-Ala was finished.

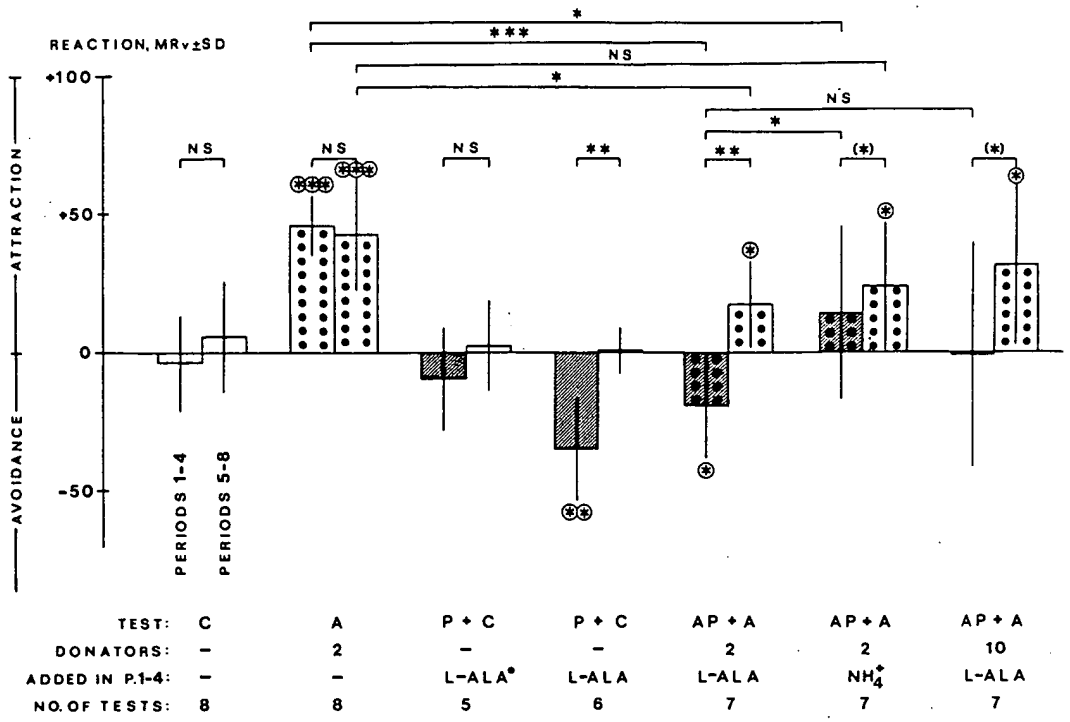

Fig. 3. The preference–avoidance behaviour of juvenile Arctic charr to water scented by conspecifics, with and without the simultaneous supply of L-Ala or ammonium chloride. The concentration of L-Ala and ammonium chloride in the test area was 4.6 and 5.6 μM, respectively. L-Ala* means 0.46 μM of that agent. Significance levels using paired t-test and Student's t-test were as stated in Fig. 2. Further explanation in Fig. 1.

At the supply of 5.6 μM NH_4^+ together with conspecific odour from two specimens, the total reaction was indifferent with two out of seven *Rv*:*s* negatively signed. Without any further ammonium chloride supply in test periods 5–8, attraction to "pure" conspecific odour appeared anew ($P < 0.05$). The response to 4.6 μM L-Ala added to odour from two donors differed significantly from the corresponding value when 5.6 μM ammonium chloride was added (Fig. 3).

The reactions to water scented by two charr in AP + A tests, with supply of L-Ala or ammonium chloride, were both significantly lowered as compared with the results from corresponding "pure" attraction tests ($P < 0.001$ and $P < 0.05$). A significant difference was still present in periods 5–8 since the supply of L-Ala to water scented by two donors had ceased ($P < 0.05$). No significant difference was observed on the final periods, 5–8, between tests with two and 10 donors, respectively, since the supply of L-Ala was stopped.

DISCUSSION

The addition of ammonium chloride or L-alanine can reduce or totally eliminate the attractive effect of conspecific scent in Arctic charr. This indicates that excretion products such as ammonium/ammonia and amino acids can be responsible for the repulsive effect of water presumably containing fish-attractants which have been reported (Le Martret and Saglio, 1982; Stabell, 1982). In the present study the repellent effect of L-Ala was stronger than that of ammonium chloride. The former agent gave avoidance, the latter compound gave merely an indifferent response. The concentration of attractants seemed to have an influence on the repulsive effect as 4.6 μM L-Ala gave a significant avoidance with two, but an indifferent reaction with 10 donors. In the latter case two *Rv*:*s* were highly positive, i.e. strong attraction to scented water. Nothing similar to this was observed in the tests with two donors. The high diversity in response during the supply of L-Ala in attraction tests using 10 donors implies that the attractive force dominates over the repulsive effect in some specimens. It seems to be a competition between the attractive force of conspecific attractants and the negative force of the nitrogenous substances tested, i.e. L-Ala and ammonium chloride. And with increasing concentration of attractant(s) in proportion to the repellent(s) the attractiveness will probably dominate the more. The addition of 5.6 μM ammonium chloride did not affect the strength of attraction to scent from 20 donors, while the same concentration of ammonium chloride eliminated the attraction to water conditioned by two donors only. Water scented both by two as well as by 10 charr is *per se* strongly attractive (c.f. Olsén and Höglund, 1985). The results support the statement by Olsén (1986b) that the attractive effect of charr scented water cannot be attributed to commonly occurring amino acids and ammonia/ammonium, although these substances may instantaneously induce up-stream movement and food search behaviour in the same species (Olsén *et al.*, 1986). The attractants may rather be sought among other agents, possibly steroids as suggested by Selset (1980), Selset and

Døving (1980), Algranati and Perlmutter (1981) and Stabell *et al.* (1982). The repulsive effects of mixtures of amino acids, alanine and ammonia/ammonium as shown in the present tests and discussed in a previous article (Olsén, 1986b) may instead be connected to the fishes' recognition of enhanced concentrations of nitrogenous metabolites in comparison with conspecific attractants due to crowding or other stress giving rise to an enhanced secretion of skin mucous, cellular debris therein, and also coupled to an increased membrane permeability (e.g. Fletcher, 1981; Peters *et al.*, 1981; Hunn, 1982; Wechsler, 1984). Then an enhanced loss of amino acids may occur as observed in crustaceans during stress (e.g. Gardner and Miller, 1981; Bengtsson, 1982). Skin mucous and urine are potent olfactory stimuli in fish, due, at least in part to free amino acids (Døving *et al.*, 1973; Stabell and Selset, 1980; Fisknes and Døving, 1982; Ogata *et al.*, 1983; Hara *et al.*, 1984). The present results may be in keeping with the proposal that organic wastes and/or changed concentrations of respiratory gases act as modifiers of the internal structure of fish schools (McFarland and Moss, 1967; Moss and McFarland, 1970).

One question which emerges from the present and the previous studies (e.g. Olsén, 1986a, b) concerns the extent to which excretion products and fish "pheromones" from fish farming activities influence the behaviour of wild fish populations.

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Feeding Behaviour in Adult Rainbow Trout and Atlantic Salmon Parr, Elicited by Chemical Fractions and Mixtures of Compounds Identified in Shrimp Extract

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(Accepted 13 January 1987)

ABSTRACT

Mearns, K.J., Ellingsen, O.F., Døving, K.B. and Helmer, S., 1987. Feeding behaviour in adult rainbow trout and Atlantic salmon parr, elicited by chemical fractions and mixtures of compounds identified in shrimp extract. *Aquaculture*, 64: 47–63.

A bio-assay method was used to assess the feeding response of adult rainbow trout, *Salmo gairdneri* R., and Atlantic salmon parr, *Salmo salar*, to chemical fractions and mixtures of compounds identified and quantified in an aqueous extract of shrimp. Purified agar was flavoured with (A) aqueous extract, (B) methanol fraction, (C) basic/amphoteric faction, (D) acidic fraction, (E) neutral fraction, (F) no flavouring, (G) amino acids, (H) nucleotides, (I) adenosine, inosine, hypoxanthine, creatine and glucose, (J) trimethylamine, trimethylamineoxide, glycine betaine, and homarine. Each gel was presented over a short-term feeding period and observations were made of the number of gel pieces swallowed, the number of gel pieces ejected and other behaviour patterns associated with feeding, e.g, increased activity and "tasting". The results indicate that both species found A and B to be the most palatable whereas E and F were the least palatable (as measured by number of swallows and number of pieces ejected). The rainbow trout ate almost all the gels presented whereas the salmon parr ate only A and B. The various fractions and chemical mixtures were rated as feeding stimulants, incitants and deterrents on the basis of the behavioural data recorded for each species and the classification of Lindstedt (1971).

INTRODUCTION

The role of chemical cues in the release of feeding behaviour in salmonids has yet to be fully investigated. In other species of fish, e.g., the cod (*Gadus morhua*), specific chemical components are responsible for eliciting various behaviour patterns used in the detection and identification of food (Ellingsen and Døving, 1986). Unlike cod, salmonids tend to rely on vision to detect their prey; however, the final acceptance of a food item is usually determined by taste. The identification of feeding stimulants for salmonids, which are com-

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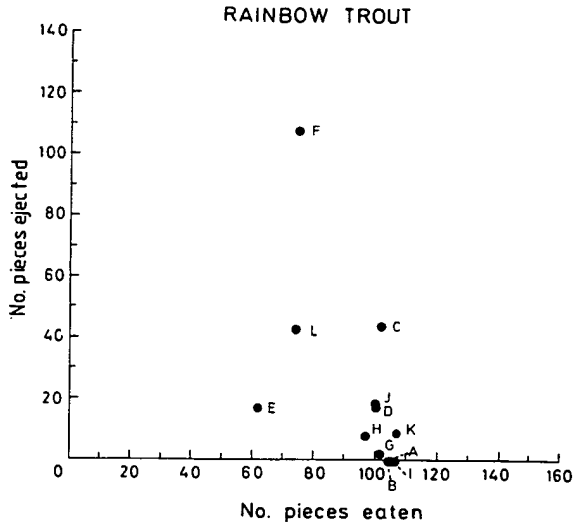

Fig. 3. Palatability of each gel for the rainbow trout, plotted as number of gel pieces ejected vs. number of pieces eaten. The contents of each gel are given in Table 1.

involved holding the gel pieces in the buccal cavity and engaging in small, rapid jaw movements, was seen particularly in response to gel C, the basic/amphoteric fraction, and gel F, the blank. The response of the fish to gel C was interesting. Three of the trout had extremely high ejection rates, accompanied by "tasting", "coughing" and even regurgitation of the gel pieces after feeding. All the gel pieces, however, where eventually swallowed.

There were no significant differences between gels G–L regarding number of pieces swallowed ($X_r^2 = 6.34$), i.e., all gels were eaten equally well. The number of gel pieces ejected, however, differed significantly at the 0.05 level ($X_r^2 = 12.4$), with the blank being ejected more frequently than the others. Increased activity was only observed in response to gel K, the protein-free extract, and "tasting" was observed for gels L (blank), H (nucleotides) and I (the mixture of adenosine, inosine, hypoxanthine, creatine and glucose). The fish appeared hesitant about accepting gel G (the amino acid mixture), i.e., they did not consume it immediately as they did with the other gels, but nearly all the pieces were eventually eaten.

Atlantic salmon parr

Fig. 4 shows the percentage of gel pieces of ech flavouring eaten by the salmon parr. In contrast to the rainbow trout, the Atlantic salmon parr ate only gel A (stock solution) and gel B (protein-free extract), with 67 and 40% being consumed, respectively. Many of the gels containing other flavourings were not eaten at all. The only others to elicit a slight feeding response were gels C

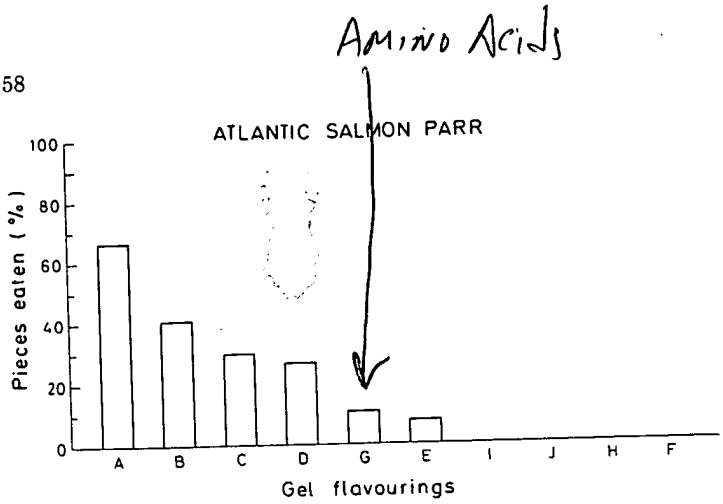

Fig. 4. Percentage of gel pieces eaten by the Atlantic salmon parr. The contents of each gel are given in Table 1.

(basic/amphoteric compounds), D (acidic compounds) and G (mixture of amino acids).

Fig. 5 shows the palatability of each gel flavouring in terms of number of pieces swallowed plotted against number ejected. As can be seen from the cluster of points on the *y* axis most of the flavourings induced a high ejection rate and little swallowing, i.e., they were highly unpalatable for the fish. Even gel A (stock solution) was not well accepted, although it was taken into the mouth most often. Gels B and K (both protein-free extracts) induced the same level of swallowing but the rate of ejection declined on the second presentation (gel K). Gels D and C were both highly ejected, although unlike most gels they did induce some swallowing.

Statistical analysis of the data showed significant differences between gels A–F for number of pieces swallowed ($X_r^2 = 39.65$, $P < 0.001$) but no significant differences for number of pieces ejected ($X_r^2 = 7.45$). Stock solution (A) was eaten more often than E (neutral compounds) and F (blank). The protein-free extract (B) was also eaten more often than the blank. The fish were observed to increase their level of activity after they had been fed gels A, B and C and "tasting" was observed in response to gels A, B and C. For gels G–L, only K, the protein-free extract, and G, the amino acid mixture, were eaten to any extent at all and no differences were found beween the gels with regard to number of pieces expelled. The fish only increased their level of activity in response to gel K and to a lesser extent gel G, and "tasting" was only observed in response to gel K.

DISCUSSION

In this bio-assay, the observation of both rate of ejection and swallowing of flavoured gel pieces has given us a clearer indication of the palatability of various chemical mixtures for adult rainbow trout and Atlantic salmon parr.

ORLando, FL
Tallahassee, FL
Newport News, VA
Norfolk, VA

Use this stuff, and fish Gotta Bite

Scientist unveils new concoction

By Don Wilson
Orlando Sentinel

ORLANDO, Fla.

You've probably never heard of John Caprio, but the Louisiana scientist will soon eclipse Einstein — with one part of the world's population, at least.

The Louisiana State University researcher has perfected a solution that triggers an uncontrollable, automatic biting response in fish.

It's the ultimate bait — something no fish can resist.

To the fishing fraternity it's the same as a golfer's can't-miss ball with a built-in inertial guidance system.

The concoction, a combination of amino acids, promises to change forever the way the millions of anglers approach their pastime.

Forget the fishing forecasts. Scrap those solar-lunar tables.

That tackle box stuffed to overflowing with every conceivable fish-fooler? Trash it.

All you'll need will be a handful of basic lures (or just bare hooks and cotton balls) and a bottle of Gotta Bite.

That's the name with which Caprio and research partner Tine Valentincic have christened their discovery.

Don't start for your local tackle store yet.

Production and marketing of the elixir is still in the future. And there's another catch — it works, but mainly on catfish.

Caprio said he focused his research on that species because it was the ideal guinea pig.

"Catfish literally are nothing but swimming tongues," he said. "A catfish is a chemical sensory computer and one just 6 inches long has a couple of hundred thousand taste buds along the outside of its body."

To find out just which amino acids — there are 21 of them — in what concentrations produce what response, Caprio and Valentincic conducted what amounted to underwater EEGs. They wired a catfish's skull to transmit the brain's electrical responses to a computer.

Then they tried dripping various combinations of the amino acids into the aquarium tank and observing both the electrical and physical reactions.

"When we saw the responses, it really got us," Caprio said. "Tine is a fisherman, and he said he'd never seen anything like this."

In weak concentrations, the solution caused a dozing catfish to start twitching and searching its tank.

"When we increased the concentration, he had to bite — anything. He tried to bite the aquarium glass, picked up stones in his mouth — he just had to bite," Caprio said.

Knowing that what works in the lab can flop in the real world, the pair took their discovery along on a fishing trip to the Gulf of Mexico.

Using nothing but a bare hook and a small cotton ball saturated with Gotta Bite, Caprio said, "we caught drum and other species as well as catfish."

He thinks even in its present form, the solution would "catch the attention of a bass or trout."

All that would be needed would be for an interested company to finance a year's research, estimated to cost $100,000, he said.

Several have sniffed, but one Louisiana firm is ready to sign for the rights to make and market the formula, said Don Pennington, director of LSU's technology transfer office.

Pennington said the first form of Gotta Bite to be marketed will be as an additive to catfish feed used by fish farmers. Apparently, when fish are transferred from one farm to another, they lose their appetites in strange environments and many weaken and die of disease. The Gotta Bite would end that appetite loss, he said.

He said he's had some interest from other firms wanting to find versions of the formula that work on bass, walleye and other sport fish.

"They've offered to finance the research, but they haven't put any money on the table yet," Pennington said.

VEKTOR FISH & GAME ACTIVITY TABLES™

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Tables indicate peak fish and game feeding and migration times. Major periods can bracket the peak by an hour before—hour after. Minor peaks—½ hour before and after. Standard time.

		AM		PM	
		Minor	Major	Minor	Major
Feb.	3	— —	6:46	12:01	5:18
	4	7:06	12:13	12:32	5:56
	5	7:25	12:39	1:06	6:35
	6	6:43	1:04	7:18	1:41
	7	8:02	1:29	8:10	2:22
	8	1:54	8:22	9:20	3:12
	9	2:19	8:45	11:10	4:14
	10	2:45	9:15	— —	5:30

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NATIONAL Publication

Biology

They gotta have it

Researchers studying the physiological mechanisms behind taste and smell in fish have netted an unanticipated catch: a simple compound that gives catfish an uncontrollable urge to bite.

The scientists say anglers might apply the substance — whimsically named "Gotta Bite" — to lures to make gamefish all but leap into the boat. They also predict that fish farmers could use the appetite-stimulating chemical to grow bigger fish in less time.

Gotta Bite's discoverers — physiologist John Caprio of Louisiana State University in Baton Rouge, and Slovenian animal behaviorist Tine Valentincic — were examining how catfish sense amino acids when they stumbled upon their serendipitous finding. In the December CHEMICAL SENSES, they report that catfish taste but do not smell Gotta Bite, which is a blend of several amino acids, the building blocks that combine to form proteins.

In 1977, Caprio discovered that catfish, unlike most fish, do not taste the same amino acids they smell, or smell the same amino acids they taste (SN: 5/21/77, p.332).

Taste researchers study catfish because these animals have an exquisite sense of taste. "Catfish are basically swimming tongues," says Caprio, "so they are ideal models for our research."

But even though catfish usually respond heartily to good tastes, Caprio says he and Valentincic were shocked by the fish's initial response to Gotta Bite. At low concentrations of the substance, the fish began a series of rapid turns, as if searching for the source of the yummy flavor. As the concentration of Gotta Bite increased, the fish flew into a feeding frenzy — champing at the water, gobbling up and spitting out small rocks, and snapping at the glass walls of the aquarium.

Louisiana State University has applied for patents for using Gotta Bite to coat fishing lures and as a diet supplement for farmed fish. Caprio cautions that he and Valentincic have not yet tested the substance outside the laboratory. However, he says, "if it works in the field half as good as it works in the lab, it's going to be something."

And you thought *you* hated mornings

Many people start each day with a steaming mug of coffee to sweep away the last cobwebs of sleep. But coffee just doesn't pack a big enough punch for the male members of a remote Amazonian tribe called the Achuar Jivaro, report two ethnobotanists from Washington University in St. Louis.

Walter H. Lewis and Memory Elvin-Lewis found that Achuar Jivaro tribesmen — who live in the Amazonian regions of Peru and Ecuador — each morning quaff an herbal tea that contains the caffeine equivalent of five cups of coffee. But even more interestingly, the two researchers discovered that the men routinely vomit up most of the tea in order to avoid caffeine-overdose symptoms such as headache, profuse sweating and a bad case of the jitters.

The daily vomiting, or emesis, "is simply part of [an Achuar Jivaro] macho ritual, passed down through the ages," says Lewis. "The tea is so pleasing that they overindulge, vomit to rid themselves of the excess caffeine, then go about their business," he says.

The Achuar Jivaro make their jolting beverage from the leaves of a South American holly, *Ilex guayusa*, which contains the highest percentage of caffeine by dry weight of any plant in the world, Lewis says.

Through biochemical studies, the Washington University team determined that the holly does not contain any naturally occurring emetic, which would have explained the tribesmen's vomiting.

Biomedicine

Ulcer drugs make a drink more potent

For some ulcer sufferers, a sip of the grape or the grain may pack a surprisingly strong punch. Clinical studies suggest that two commonly prescribed ulcer medications can significantly increase alcohol's intoxicating effects.

In 1989, researchers led by Charles S. Lieber of the Mount Sinai School of Medicine in New York City found that people taking the ulcer drug cimetidine (Tagamet) may become intoxicated even if they drink only small amounts of liquor. Now, the same team reports that another commonly prescribed ulcer drug, ranitidine (Zantac), also boosts the effects of liquor.

In the Jan. 1 JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION, Lieber and his colleagues describe a study in which they gave 20 healthy men breakfast followed by a glass of orange juice spiked with an alcohol dose equivalent to 1½ glasses of wine or beer. To establish a baseline, the researchers measured blood-alcohol concentrations after the volunteers consumed the drink. Then, for the next week, they gave eight men 300 milligrams per day of ranitidine, six men 1,000 milligrams per day of cimetidine and six men 40 milligrams per day of famotidine (Pepcid), another ulcer medication.

At the end of the week, the researchers again gave the volunteers an orange juice spiked with alcohol. Ranitidine and cimetidine treatment boosted the group's mean peak blood-alcohol concentration by 34 and 92 percent, respectively, compared with baseline. Famotidine had no significant effect on blood-alcohol concentration.

The researchers can't completely explain the alcohol-enhancing ability of the two drugs. Lieber notes, however, that both cimetidine and ranitidine belong to a class of drugs that inhibit gastric alcohol dehydrogenase, an enzyme that metabolizes alcohol. When the enzyme activity is slowed, more alcohol reaches the bloodstream, he suggests. Thus, people who drink while taking these drugs may run a risk of impaired functioning, which could make driving a car and other attention-oriented tasks hazardous, Lieber says.

Asbestos linked to colon polyps

A new study indicates that people exposed to significant amounts of asbestos may face an increased risk of colon polyps, small, grape-shaped growths that can become malignant.

Although epidemiologists have linked the mineral asbestos to the development of colon cancer in the past, this is the first time scientists have shown an association between asbestos and colon polyps. The finding suggests that asbestos may act very early to trigger the wart-like polyps, says epidemiologist Alfred I. Neugut of Columbia University's School of Public Health.

He and his colleagues identified patients who had undergone colonoscopy at two New York City medical centers between April 1986 and March 1988. After reviewing medical charts, the researchers discovered 51 men with colon cancer, 153 men with colon polyps and 195 who showed no sign of colon cancer or polyps. They asked the men a series of questions, including questions about their exposure to asbestos, the fibrous mineral widely used as an insulating and fireproofing material.

A statistical analysis revealed that men who reported heavy exposure to asbestos had a greater risk of colon cancer or polyps than did men who reported little or no asbestos exposure. The team describes its results in the Dec. 18 JOURNAL OF THE NATIONAL CANCER INSTITUTE.

Neugut cautions that these findings remain very preliminary. The researchers identified only 12 men with significant asbestos exposure — a group too small to yield definitive results, he says. A larger study would help strengthen the evidence linking asbestos to colon polyps, he notes.

Jacksonville, FL. March 8/92

Catfish can't seem to resist Gotta Bite

OPINION

Joe Julavits

Outdoors editor

Amid the bubbling flasks and foul-smelling test tubes in the Louisiana State University campus lab, there's a scientist at work who is on to something big.

Something huge.

We could be talking milestone. Like the internal combustion engine. Wright Brothers. The wheel. Hey, this thing could be bigger than the plastic worm.

It's called Gotta Bite, the absolute can't-miss, take-it-to-the bank ultimate fish bait. Squirt a few drops on a cottonball, and your tackle box becomes extinct. You'll never buy another lure. Live bait? Who needs it.

This is no ordinary fish scent dreamed up by some tackle company marketing whiz or some farmer who accidentally spat tobacco juice on his wriggler. This one's got science on its side, and just enough mystery to make it sound legit.

The man behind the marvel, research scientist John Caprio, doesn't know much about fishing. But he has found out what makes them bite, and if he can successfully transfer that knowledge into a little jar of Gotta Bite, he can buy his own lab.

Caprio's research began innocently enough a couple of years ago while studying neurological responses in animal and fish species. He wired a catfish's brain — that in itself is news since many anglers didn't think the dimwitted fish had one — and observed its responses to various doses of amino acids introduced into an aquarium tank.

When the acid reached a certain concentration, the catfish went into a biting frenzy. The professor and his assistants knew they had hit upon something. After further tests, they brought their discovery to Don Pennington, an avid bass fisherman who runs the patenting and licensing department at LSU.

Pennington witnessed tests with more catfish and saw the full potential in the experiment.

"As soon as that liquid hits the water, they immediately start snapping at everything in the aquarium," Pennington said. "You can throw a lead weight in there and they'll bite it."

Controlled field tests were ordered and the ravenous catfish responded as they had in the aquarium. Two ponds were used in the study, with 1,000 catfish fed regular food pellets in one, and 1,000 others put on the "LSU diet" in the second pond.

A strange byproduct of the tests was that the fish in Pond 1 all grew to various sizes, while the LSU fish were all the same exact size. "We don't know why," said Pennington.

Convinced that it worked, at least on catfish, the researchers gave the concoction a catchy name, and recently applied for two patents. One covers the top-secret composition of materials used in the involuntary stimulation of catfish and the other is for the use of Gotta Bite as a commercial bait.

Pennington is currently hammering out a licensing contract with a Louisiana firm ("We discover things, we don't develop them," he says), with his immediate sights set on the catfish farming industry.

But the sport-fishing brethren also have caught the scent of Gotta Bite, and they're hooked.

"I've gotten calls from every major bait distributor in the country," Pennington said. "I get probably 10 calls a week from fishermen about it."

Pennington said Gotta Bite could be on the shelves in six months, depending on the patent process. But he stresses that it has been properly field-tested only for catfish.

Fishermen interested in a surefire bait for other species will have to wait on Prof. Caprio, who spent two years on the catfish project. Among the subjects of his newest research is the ever-popular largemouth bass.

If Caprio unlocks the secret of what triggers bass or other gamefish to bite, he'll be the most popular man in Baton Rouge.

"Professors are rather naive relative to the real commercial world," Pennington said. "My job at the university is to make them rich.

"Inventors get 40 percent of the royalties from any licensing agreement. If it's worth $10 million, [Caprio] gets four million bucks."

That ain't fish feed.

Cedar Rapids, IOWA

OUTDOOR

'Gotta Bite' sends catfish into feeding frenzy

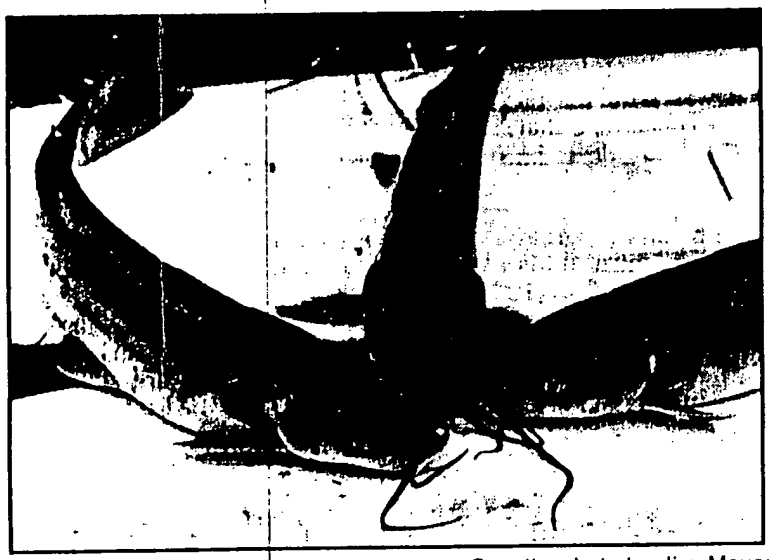
Even sleeping fish go crazy to hit LSU bait

BATON ROUGE, La.— While searching for scientific answers to questions of taste and smell, an LSU-based research team has discovered what could be developed as the ultimate, sure-fire catfish bait.

LSU physiologist John Caprio and Slovenian (Yugoslavian) animal behaviorist Tine Valentincic, have developed a compound that sends catfish literally chomping at the bit.

"It gives them an uncontrollable desire to bite," says Caprio. They have named it — what else but, "Gotta Bite."

LSU is patenting Gotta Bite for any possible sports-fishing lure applications and as a food additive to make pond-raised catfish eat more so they can get fatter faster.



Gazette photo by Jim Mayer

These Iowa channel catfish won't have a chance with the new bait Louisiana State University has discovered.

"Gotta Bite is just an incidental spinoff of our basic research which benefits science and medicine," he says. "It still needs a lot of developmental work."

"Research into how sensory systems of taste and smell operate in catfish is basic to our understanding of how these senses work in all vertebrates, including humans," he says.

Caprio said he uses catfish as a laboratory model because — from whisker to tail fin — it is a swimming mass of taste buds and has a highly developed brain and nervous system.

BEHIND GOTTA BITE is some 20 years of basic science research by Caprio, initially heralded by "Nature," the pre-eminent British journal of science that in 1977 published the LSU scientist's landmark findings on the catfish's unique sensory mechanisms.

Caprio was the first to report that the sense of taste in catfish is highly sensitive to amino acids, the building blocks of proteins. Since then, he has worked to find the amino acid combinations that work best.

How Gotta Bite works is no fisherman's tale. LSU has it all on video.

Picture a catfish resting quietly on the bottom of an aquarium while Gotta Bite is squirted into the water. Suddenly the fish bursts from its slumber and rises in excitement, flipping wildly around the tank.

On the video, viewers hear researchers counting the 90-degree turns — one almost every second — the catfish makes in a feeding frenzy. It chomps, chomps, chomps at the water. It gobbles up and spits out aquarium rocks and even tries to munch out on the aquarium's glass walls.

Valentincic successfully trained the catfish to distinguish between compounds and found that they responded to Gotta Bite more fervently — like Pavlov's dog — with a system of rewards.

Fig 1

RESPONSES OF CHANNEL CATFISH TO AMINO ACIDS

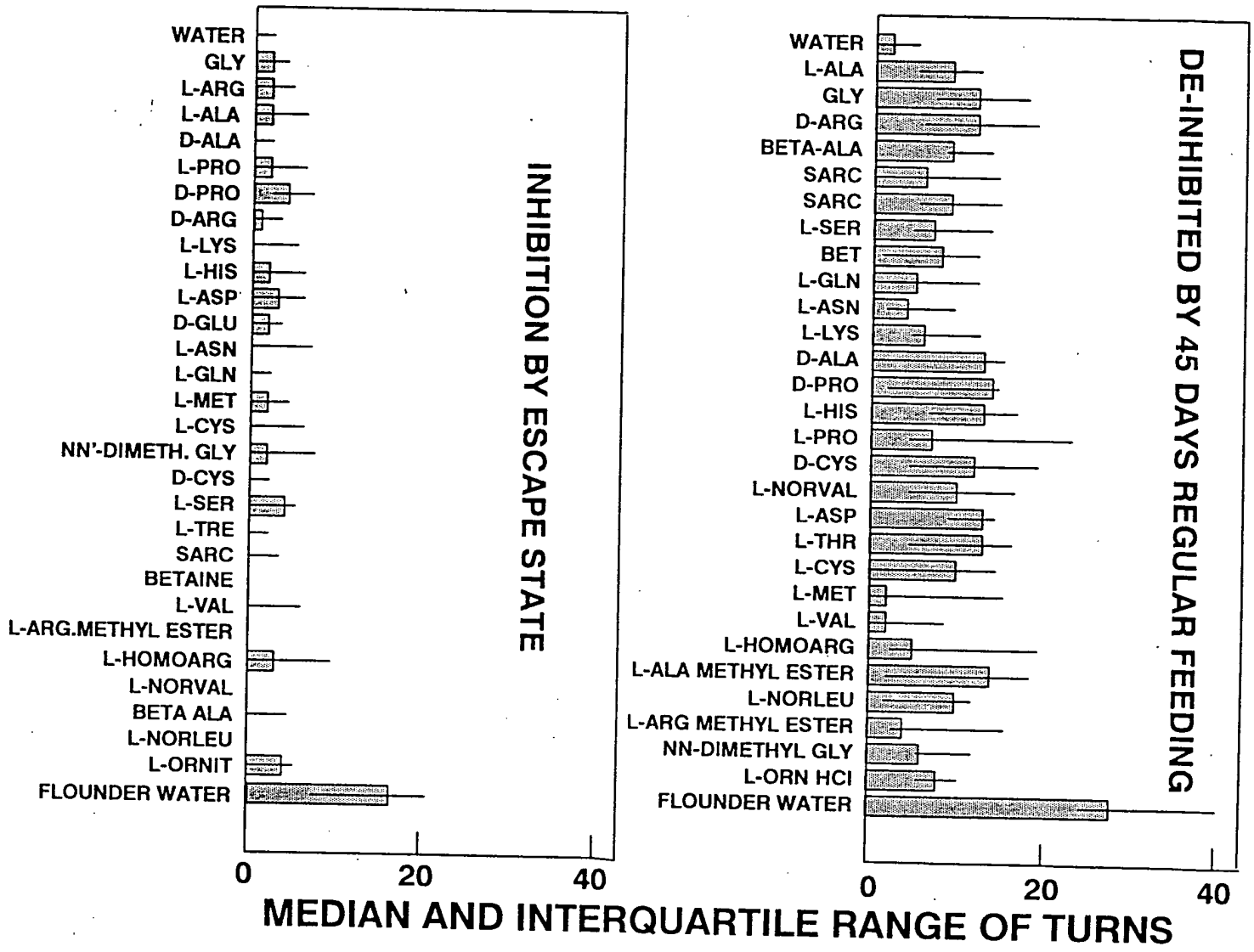


Fig 2

BITING AND SNAPPING BEHAVIOR TO AMINO ACIDS IN ANOSMIC CHANNEL CATFISH

CONCENTRATIONS: INJECTED = 10mM; EXPECTED CONTACT = [O.OO1-O.O1mM]

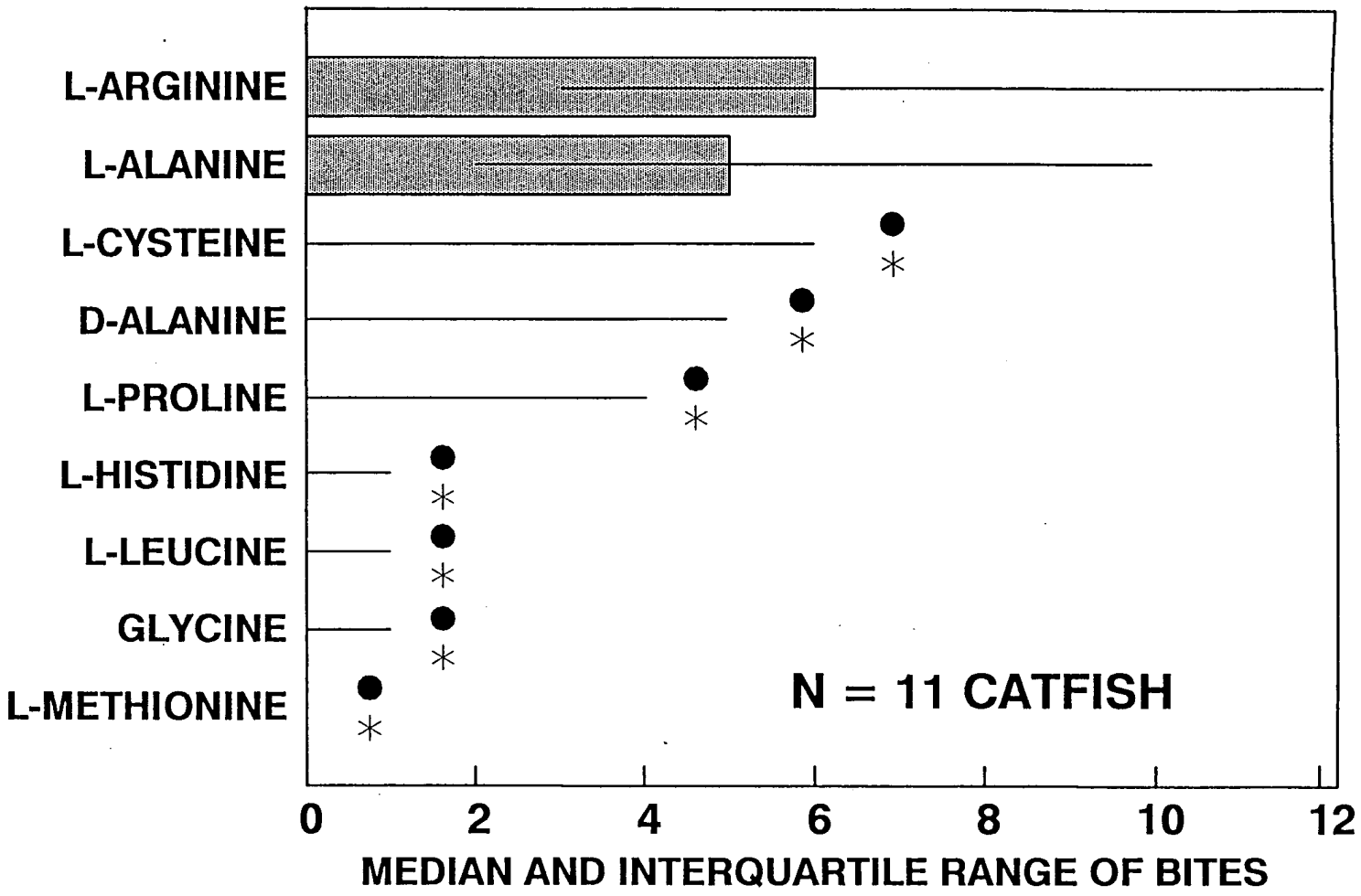


Fig 3

SNAPPING RESPONSES TO NEUTRAL AMINO ACIDS IN ANOSMIC CHANNEL CATFISH

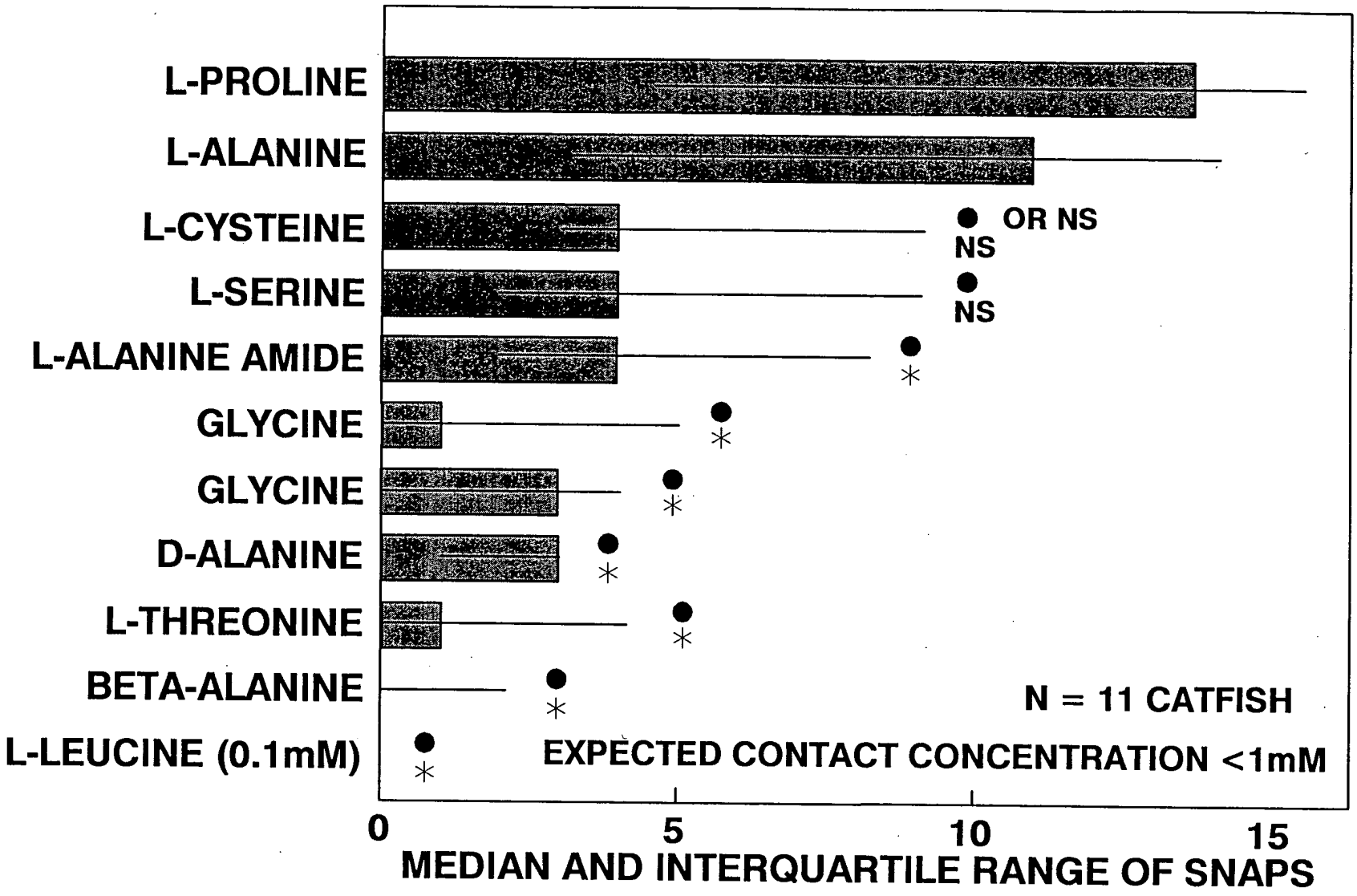


FIG. 1 NAIVE CHANNEL CATFISH WERE TESTED FOR THEIR RESPONSIVENESS (FOOD SEARCH SWIM ACTIVITY) TO SINGLE AMINO ACIDS AFTER > 15-30 DAYS (LEFT) AND > 45 DAYS (RIGHT). NOTE THE INCREASE IN RESPONSIVENESS (NUMBER OF > 90 DEGREE TURNS) TO AMINO ACIDS AND RELATED COMPOUNDS BY CHANNEL CATFISH SUBSEQUENT TO THE ADDITIONAL DAYS OF FEEDING WHICH IS CORRELATED TO A REDUCTION IN THE TENDENCY FOR ESCAPE BEHAVIOR.

FIG. 2 BITING BEHAVIOR CAN BE INITIATED SOLELY BY THE TASTE SYSTEM. ALTHOUGH AMINO ACID DISCRIMINATION LEARNING REQUIRED AN INTACT OLFACTORY ORGAN, ANOSMIC CHANNEL CATFISH EXHIBIT BITING RESPONSES TO CHEMICAL PLUMES CONTAINING MICROMOLAR TO MILLIMOLAR AMOUNTS OF EITHER L-ALA OR L-ARG; OTHER AMINO ACIDS ARE SIGNIFICANTLY ($p<0.05$) LESS EFFECTIVE KEY STIMULI FOR THE BITING RESPONSE IN THIS RANGE OF CONCENTRATIONS. DOTS INDICATE A SIGNIFICANT DIFFERENCE ($p<0.05$; WILCOXON TEST) FROM THE RESPONSE TO L-ARGININE; ASTERISKS SIGNIFY A SIGNIFICANT DIFFERENCE FROM THE RESPONSE TO L-ALANINE.

FIG. 3 ABOVE 1 MM, L-PROLINE AND L-ALANINE ARE THE MOST EFFECTIVE STIMULI AT RELEASING THE BITING RESPONSE. DOTS INDICATE A SIGNIFICANT DIFFERENCE ($p<0.05$; WILCOXON TEST) FROM THE RESPONSE TO L-PROLINE; ASTERISKS SIGNIFY A SIGNIFICANT DIFFERENCE FROM THE RESPONSE TO L-ALANINE.